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Federal Working Group on Pest Management, Rockville, Md

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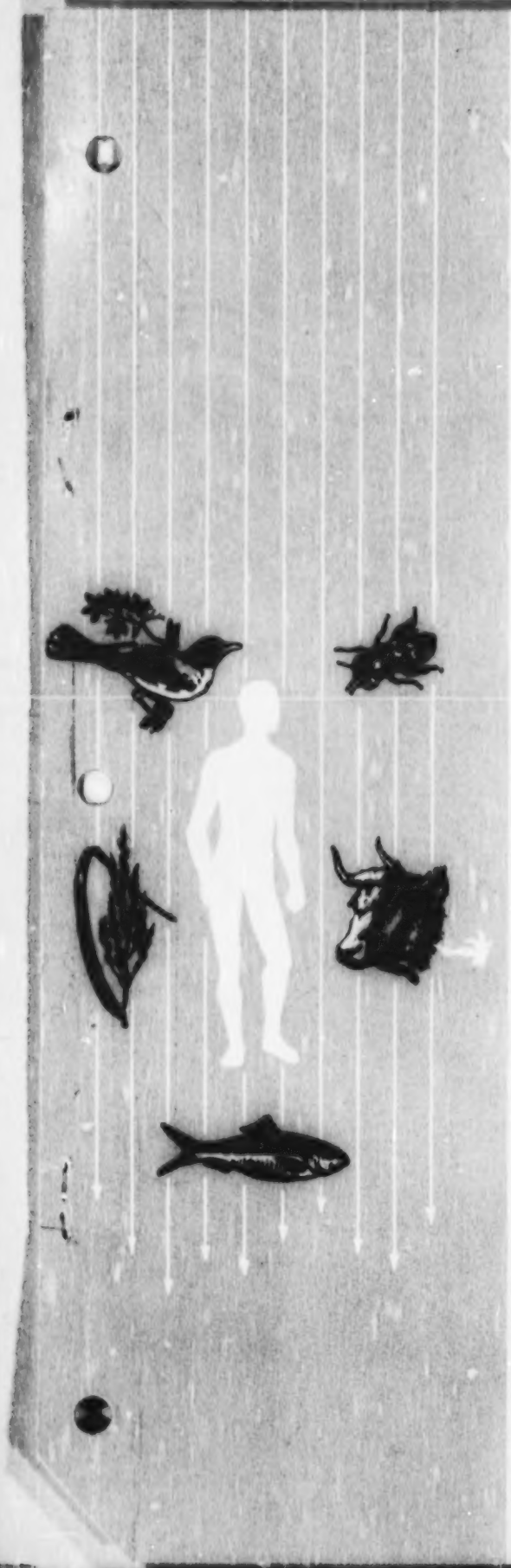

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GUIDELINES ON ANALYTICAL METHODOLOGY FOR PESTICIDE RESIDUE MONITORING

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FEDERAL WORKING GROUP ON PEST MANAGEMENT
WASHINGTON, D.C. 20460

JUNE 1975

FEDERAL WORKING GROUP ON PEST MANAGEMENT*

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J. V. Smith
J. R. Wessel

Department of the Interior
W. H. Stickel

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C. J. Frederickson
B. L. Long

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J. Lewis, *Acting Chairman*
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J. L. Brooks
E. J. Burger, Jr.

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J. H. Caro, *Agricultural Research Service*
E. L. Cox, *Agricultural Research Service*
L. Heffner, *Extension Service*
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M. S. Schechter, *Agricultural Research Service*
H. M. Trabosh, *Animal and Plant Health Inspection Service*
R. L. Williamson, *Animal and Plant Health Inspection Service*

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J. R. Wessel, *Food and Drug Administration*

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D. H. White, *Fish and Wildlife Service*

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J. H. Vinopal, *Department of the Army*

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D. S. Dennis, *Office of Pesticide Programs*
R. G. Heath, *Office of Pesticide Programs*
F. W. Kutz, *Office of Pesticide Programs*
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D. L. Worf, *National Environmental Research Center, N.C.*

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SECRETARIAT

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A. Inglis, *Assistant Executive Secretary*

* Responsible to the Council on Environmental Quality.

GUIDELINES ON ANALYTICAL METHODOLOGY FOR PESTICIDE RESIDUE MONITORING

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Dr. D. G. Ballinger, ORD, EPA
Mr. J. A. Burke, FDA, DHEW
Mr. J. H. Caro, ARS, USDA
Dr. H. F. Enos, ORD, EPA
Dr. K. R. Hill, ARS, USDA
Mr. W. L. Reichel, FWS, USDI
Dr. E. A. Woolson, ARS, USDA
Mr. M. L. Yates, GS, USDI

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Dr. D. L. Stalling, FWS, USDI
Dr. H. Tai, OPP, EPA

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CHAPTER I - Introduction

Considerable research effort in recent years has gone into a variety of alternative pest control methods such as the use of attractants, repellents, juvenile and other insect hormones or growth regulators, pheromones, insect sterility by radiation or chemosterilants, pest resistant crops, parasites and predators, pathogenic agents, etc. However, the use of chemical pesticides is still the mainstay in large-scale control of most insects, weeds, fungi and other pests of economic or public health importance. In addition to widespread use of pesticides by farmers, nurserymen, pest control operators and homeowners, various agencies of the federal, state, and local governments often have large-scale programs of their own, such as for the control of mosquitoes, forest insects, grasshoppers and crickets, certain terrestrial and aquatic weeds, and rodents.

Although monitoring for pesticide residues in the environment has been going on for many years, it was not until 1964 that a National Pesticide Monitoring Program (NPMP) was organized to monitor pesticide residues in the following environmental components: food and feed, humans, fish, wildlife, estuaries, water, and soil. In 1967 the NPMP was described in the first issue of the *Pesticides Monitoring Journal* (PMJ); that journal has remained the major outlet for papers relating to the NPMP and pesticides monitoring in general. A *Chemicals Monitoring Guide for the National Pesticide Monitoring Program* was also published in the first issue of the PMJ to focus attention on the pesticides deemed to be most important for monitoring (Schechter, 1967).

The design of the original components changed over the first several years of the Program and the revision of each component was described in volume 5, number 1 of the PMJ. The *Chemicals Monitoring Guide* was also revised in this same issue (Schechter, 1971). Citations for individual programs in both the original and revised versions of the NPMP are given in the list of references at the end of this chapter.

Rapid advances in analytical chemistry, instrumentation, and techniques are being made almost daily for the determination of pesticide residues, their metabolites and degradation products in environmental samples. In addition, modifications are often made in analytical methods in attempts to improve, to simplify or adapt them to available instruments, or to enable more samples to be run in a shorter time. For these reasons, the Monitoring Panel of the Federal Working Group on Pest Management has prepared these *Guidelines* as an aid to analytical laboratories in the selection of methods, in making appropriate modifications, and in producing and reporting valid monitoring data.

Many of the analytical methods, such as those for organochlorine or organophosphorus pesticides, are multiresidue methods that will detect a number of pesticides and their metabolic or degradation products. Great progress has been made in recent years in standardizing such methods by collaborative studies under auspices of the Association of Official Analytical Chemists (AOAC). The reader should refer to methods published in *Official Methods of Analysis of the Association of Official Analytical Chemists* (Horwitz, 1975), the *Journal of the Association of Official Analytical Chemists*, and in the Food and Drug Administration's (FDA) *Pesticide Analytical Manual*. A comprehensive review of methodology used by FDA for organochlorine pesticide residues in foods has been published (Burke, 1971).

The *Guidelines* are written in a general outline form in order to cover the wide variety of methods, techniques, and instruments employed in pesticide residue analysis while sparing the reader cumbersome details. The main topics generally follow the steps or considerations usually involved in analysis of monitoring-type samples, namely: Safety; Sampling; Storage of Samples; Solvents and Reagents; Extraction; Clean-up; Detection and Quantitation; Confirmation of Residue Identity; Sensitivity and Limit of Detection; Losses and Degradation of Pesticides; Statistical Evaluation and Reporting Results; Metabolites and Degradation Products; Multicomponent Pesticides and Polychlorinated Biphenyls; and Reference Standards.

These *Guidelines* draw upon the experiences of the Monitoring Panel, its Task Group on Analytical Methodology and their colleagues. Selected references on the appropriate topics are provided at the end of most chapters as a representative, but by no means exhaustive, sampling of pertinent literature. The last chapter identifies pesticide residue manuals prepared or used by various government and nongovernment entities. The last chapter additionally presents a listing of useful references not listed in previous chapters.

Although heavily slanted toward gas chromatography (GC) methods because of their widespread use, many of the considerations mentioned in these *Guidelines* would apply to other methods. Because there is such a wide variety of pesticides differing considerably in physical, chemical or biochemical characteristics such as solubility, vapor pressure, photodegradability, hydrolysis, metabolism, etc., it is impossible to make categorical statements that will hold for all types of pesticide samples under various conditions.

Identification, and especially confirmation of identity, of pesticide residues in analysis of environmental samples is one area that often causes considerable difficulty for analysts and those who review the data and results critically. With so much reliance placed on gas-,

paper-, and thin-layer-chromatography (TLC), there is always the possibility that a tentative identification made on the basis of a single chromatographic analysis might not be correct. Hence, the analyst must select enough auxiliary means of identification to satisfactorily confirm the identity of the pesticide(s) reported. Gas chromatography-mass spectrometry (GC-MS) is gaining more prominence as a reliable technique for confirmation of residue identity. Because of the importance of confirming the identity of reported residues, a special section of the *Guidelines* has been devoted to this subject.

High-pressure liquid chromatography (HPLC) is a relatively new technique now being used in an increasing number of pesticide residue methods. With improvements in column and packing technology, apparatus design, detector sensitivity, and cost, it is likely that HPLC will play an increasingly greater role in residue analysis. It offers advantages of room-temperature operation with little or no degradation of the pesticides, and excellent separations possible because of the large choice of solvents, solvent mixtures, or gradient elutions. HPLC can also be applied to nonvolatile pesticides and degradation products that cannot be analyzed by GC. This new technique offers great promise but the number of residue methods to which it is applicable, and the available experience do not warrant further comment at present.

Advances are also being made in automation. Systems are available that are capable of automatic injection of a series of samples into a gas chromatograph in the absence of an operator. Data acquisition systems enable the storage of large amounts of data with computerized printouts that greatly improve the speed and efficiency of analyses. While such systems can presently be used when the nature of the samples and pesticides to be determined is well known, a skilled analyst is required to interpret the results of multiresidue analyses in which unknowns and interferences can cause serious difficulties.

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CHAPTER II - Safety

Any laboratory analyst should use every safety precaution available in the pesticide residue laboratory. However, unique precautions are in order:

Do not breathe dangerous fumes of such solvents as benzene, chloroform, carbon tetrachloride, ethyl ether, etc.

Work in well ventilated hoods when handling volatile chemicals.

Handle toxic chemicals with protective gloves.

Use explosion-proof blenders, refrigerators, and other electrical equipment.

Use shields for protection from potentially explosive reactions.

Wear goggles, face shields or safety glasses and other protective clothing

Because large volumes of flammable solvents are often required in residue analyses, suitable storage vaults should be available for large containers and special fireproof storage lockers should be available for smaller amounts in or near the laboratories. To guard against injuries from fires, adequate fire extinguishers, fire blankets, safety fountains and showers should be readily available for emergencies. Laboratories should have two exits, and aisles should be kept clear. Any offices or desks which absolutely must be located in laboratories should at least be adjacent to an exit.

REMEMBER, SAFETY IS A PRIME CONSIDERATION IN ANY ANALYTICAL LABORATORY AND ESPECIALLY IN THE PESTICIDE RESIDUE LABORATORY.

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CHAPTER III - Sampling

Analytical results can be no more valid than the samples or sampling scheme used. Samples should truly represent the environmental component being examined and should be compatible with the goals of the monitoring program. The location of sampling sites, the sampling technique, the number of samples, and the frequency and duration of sampling should be such that the analytical results can be evaluated in a statistically satisfactory manner. The size and number of samples should enable replication of analyses and confirmation of any residues found. In some cases, it has been found advantageous to composite a number of small samples.

A separate publication, *Guidelines on Sampling and Statistical Methodologies for Ambient Pesticide Monitoring* (Monitoring Panel, FWGPM 1974) is a very useful adjunct to the present *Guidelines*. The earlier report describes procedures for sampling and storage of samples more exhaustively than does the current report.

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Sampling

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CHAPTER IV - Storage of Samples

TEMPERATURE

Samples other than water should ordinarily be stored in a freezer, preferably below 0° C. Even then, physical and chemical changes may occur in either the sample or in the residues sought. Extended storage in freezers can cause moisture to migrate to the surface of the sample and then to the freezer coils, slowly desiccating the sample. This effect may be of importance if water content affects the subsequent analysis and can affect the calculated residue concentration. Water samples should be stored slightly above freezing to avoid rupture of the container as a result of freezing.

TIME

Samples should be analyzed as quickly as possible after collection before physical and chemical changes occur. If prolonged storage is required, it may be preferable to extract the sample, remove most or all of the solvent and store the extracts at a low temperature.

When feasible, studies of the stability of residues in samples or extracts, with time and temperature of storage, should be carried out with representative pesticides and substrates. When there is doubt about the stability of residues in storage, spiked control samples or extracts should be held under the same conditions as the samples or extracts.

LIGHT

Light degrades many pesticides; therefore it is advisable to protect the samples and any solutions or extracts from needless exposure. In the case of air sampling, the collection apparatus should be shielded from light during sample collection.

CONTAINERS

Avoid plastic containers, or plastic-lined caps, unless made of Teflon^{R} or other inert plastic which does not interfere in the analytical method. Aluminum foil liners for caps may tear; hence it is preferable to use Teflon^{R} sheeting or liners. If polyethylene or other plastic storage containers are used, tests should be made to determine whether there is any interference in the analysis contributed from the container; laboratories frequently have experienced such interferences.

If cans are used, they should first be checked to demonstrate the absence of materials such as oil films, lacquers, rosin from soldered joints, etc., that could interfere with analyses.

Glass containers should be used for water or liquid samples and should be thoroughly cleaned and rinsed with one or more suitable solvents such as acetone, isopropanol, or hexane, and dried before use. Pesticides can migrate to the walls of a container and be adsorbed; hence even a glass container, after the water sample is poured out, should be rinsed with solvent if the extraction is not made in the container itself.

In summary, any type of container or wrapping material should be checked before use for possible interferences in the analytical method and at the limit of detection employed in the analysis.

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CHAPTER V - Solvents and Reagents

Solvents and reagents should be free of substances that interfere with analysis or degrade the sample. To obtain low background levels and avoid spurious peaks arising from solvent impurities, it is usually necessary to employ specially purified or distilled-in-glass solvents, particularly with electron capture GC. Solvents should be checked by using them in the amounts and manner described in the method without a sample being present, concentrating them as described in the method and then testing with the particular detector prescribed. Similar precautions must be taken with reagents to make sure that they will not cause undue interference.

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Solvents and Reagents

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CHAPTER VI - Extraction

SUBSAMPLING AND PREPARATION

If required, representative subsamples should be taken after thorough mixing of the sample. Samples or subsamples should be prepared for extraction according to the procedure being followed.

Water samples containing much suspended matter or sediment may require special handling such as decantation, filtration or centrifugation after which the water and sediment can be analyzed separately, if so desired.

Plant or animal samples, either frozen or unfrozen, are frequently ground with sodium sulfate to bind the tissue moisture before extraction. Appropriate manuals or publications should be consulted for specific details for preparing plant or animal tissues.

It is generally undesirable to dry soil samples beyond the air-dry state before extraction because of possible decomposition or irreversible adsorption of pesticide residues on soil surfaces. However, soil samples should not be too wet because of particle aggregation, which can result in extraction difficulties. They should be in a damp, friable condition before extraction, with a moisture tension of 0.1-10 bars. This is equivalent to a moisture content of about 5-15% for sandy soils, 10-30% for loams. Satisfactory extraction from muck soils can be made with moisture levels as high as 85%. Moisture content should be determined on a separate portion of soil so that results can be reported on a dry-weight basis.

Sediment samples should be in the same condition as soils prior to extraction. Excess water should be removed by decantation, filtration, or centrifugation, and air-dried if necessary, until the sediment is friable

EXTRACTION EFFICIENCY

Each laboratory must choose a method which yields reasonably complete estraction and recovery of the desired residues. The extraction procedure should be proved in that laboratory unless supported by published data. Most laboratories use exhaustive extraction of samples by an appropriate solvent or solvent mixture in Soxhlet extractors since this is the most efficient extraction method known for many pesticides and substrates. Some laboratories prefer blending or tumbling of the samples with solvents for rapidity and ease of handling. Such methods can be compared to the exhaustive Soxhlet method to obtain some idea of the extraction efficiency. In the case of aggregated clay soils, tumbling with a solvent seems to be more efficient than Soxhlet extraction.

A mixture of relatively polar and nonpolar solvents (hexane-acetone, hexane-isopropanol, chloroform-methanol, etc.) is frequently necessary to thoroughly extract the pesticide residue. A nonpolar solvent alone, such as hexane, may not extract all of the residue. However, polar solvents, such as acetonitrile or acetone, are sometimes used alone. Chloroform, methylene chloride, acetonitrile, and other relatively polar solvents are frequently employed for organophosphorus and carbamate compounds, particularly, when polar metabolites are involved.

The use of radioactive-labeled pesticides applied to or incorporated in plants, animals, soils or other substrates is the best way to obtain samples for checking extraction efficiency. This technique, also useful for metabolism investigations, is generally feasible only in specially equipped laboratories and in special studies carefully designed to evaluate extraction efficiency of related procedures.

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CHAPTER VII - Cleanup

The cleanup procedures should be tested to see that they enable detection and determination of the pesticides being sought:

At the desired sensitivity or limit of detection

With reasonable recovery (preferably 85% or better)

With reasonable reduction of background interferences

With adequate separation of pesticides from non-pesticide interferences and from other pesticides.

The simplest and quickest cleanup that produces the desired results is preferable, because of the large savings in time when many samples have to be analyzed. Even though some types of samples can be analyzed with little or no cleanup, it may still be advisable to utilize cleanup steps to enhance the specificity of the analysis and to prevent undue contamination of the GC injection ports, columns, and detectors. In any case, details of the cleanup procedure should be specified fully by the developers of analytical methods. Any changes or modifications should be described explicitly, along with supporting data, by those who report results.

CLEANUP PROCEDURES

Numerous cleanup procedures are available; among them are TLC, HPLC, column-or channel-layer-chromatography, liquid-liquid partitioning, sweep-codistillation, selective photodegradation, chemical reactions such as saponification or oxidation, and reaction gas chromatography such as the use of an alkaline pre-column in the gas chromatograph. The analyst must choose the cleanup procedure or combination of procedures that will be most practical in terms of cost, time, and reagent availability. Yet, these procedures must provide extracts sufficiently clean to detect the desired pesticides at an adequate limit of detection and without undue background or spurious peak interferences.

CONCENTRATION OF SOLUTIONS

In the concentration of solutions, it is necessary to take great care during the evaporation of the last traces of solvent to avoid losses of the pesticide residue. In many instances it is actually inadvisable to remove the last traces of solvent for this reason. The use of Kuderna-Danish evaporators and micro-Snyder columns or special block heaters is recommended. Keepers, such as mineral oil or other oils of low

volatility, may be added to solutions to retard the loss of relatively volatile pesticides, especially when the last traces of solvent are to be evaporated. However, the use of keepers, while satisfactory in colorimetric procedures, is not usually desirable in the more sensitive GC methods. Rotary-vacuum evaporation may be required with heat-labile compounds.

AUTOMATED CLEANUP

Recently, techniques have been developed for automated cleanup, such as by gel permeation (gel filtration) chromatography (GPC), that offer obvious advantages in convenience and speed with which large numbers of samples can be handled. Automated cleanup techniques are expected to achieve greater popularity in the future.

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CHAPTER VIII - Detection and Quantitation

GAS CHROMATOGRAPHY

Injection

To obtain optimum quantitative results in gas chromatography the analyst must minimize errors caused by variable response of the instrument. It is desirable, in making serial determinations, to first inject a minimun of three different concentrations of the standard(s) to establish a standard curve. Linearity and reproducibility of detector response should be attained whenever possible. Injections of a single concentration of a standard solution, or a sufficient number of concentrations for a standard curve, should then be made occasionally during the period of analysis of the samples. It is good practice to have on hand, for daily or more frequent injections, a pesticide mixture which on chromatography provides an indication of the overall performance of the GC system. The proper frequency of injections of standards is a function of the stability of the instrument, the effect of the sample injections on the column, and the response of the detector. Clean extracts generally require fewer standardizations than do those with coextracted impurities that may affect the sensitivity of an electron capture detector or the performance of the GC column. An internal standard technique can be used as a means of quantitation in certain cases. An internal standard should be chosen that elutes at a retention time near the peak(s) of interest and its peak should be of about the same magnitude and preferably be well separated from the others. In most instances, it is advisable that the volumes of standard and sample solutions injected should be equal or nearly so. Syringes must be thoroughly cleaned with several solvent rinses after each injection and the septa in injection ports must be changed at regular intervals before they leak through puncture holes.

Many techniques are used for measuring the amount of liquid injected by syringe into a gas chromatograph. One satisfactory technique is to draw a small volume of pure solvent, somewhat larger than the volume of the needle, into the syringe; follow with a small air pocket; then draw in a measured volume of sample solution and follow with another air pocket. The exact volume of sample solution can then be read while it is completely in the barrel of the syringe. When injected, the small volume of pure solvent flushes the sample solution completely out of the barrel and needle into the inlet port. It is preferable to use no less than 20 percent, nor more than 80 percent, of the total volume of the syringe. Regardless of the technique used, it is imperative that both standard and sample solutions be handled in precisely the same way, and that the analyst obtain reproducibility in his injections.

Columns

Consult recent literature or relevant analytical manuals for the liquid-phase and solid-support column packing of choice for the pesticides being analyzed. Some agencies have standardized on certain columns that, based on thorough testing and experience, have proved reliable. Such columns, along with retention times and response data, are reported in the pesticide analytical manuals of FDA and EPA and in publications of other agencies.

Glass columns are preferred since they minimize decomposition and are easier to pack for optimum efficiency. Other columns should be tested very carefully before use on a particular group of environmental samples.

Columns must be conditioned at an elevated temperature, with the detector disconnected before use, to minimize bleed of the liquid phase and to obtain consistent chromatography of the compounds being measured. Injection of relatively large amounts of the pesticides to be analyzed should be done as necessary in those cases where response is improved by such treatment. Columns coated with small amounts of liquid phase (low-loaded) often give better resolution and sensitivity than heavier loaded columns, but may become contaminated more easily and are more prone to interaction of the compound being chromatographed with the solid support. For certain monitoring applications requiring special combinations of liquid phases such as 10% DC-200 plus 15% QF-1, experience has shown the heavier loading gives a long-lived column with good resolution of many pesticides.

The analyst must be particularly careful in selecting and evaluating columns since some compounds cannot be separated on some liquid phases. Typical examples of poor separation include dieldrin and *p*,*p*'-DDE, or *o*,*p*'-DDT and *p*,*p*'-DDD on methyl silicone liquid phases such as DC-200 or OV-101. It may be necessary to use two or even three columns of different liquid-phase polarities to obtain adequate separation of all compounds of interest.

The columns should not degrade the pesticides being analyzed. For example, some columns give two peaks with endrin, a pesticide readily susceptible to column interactions. This may be acceptable to some analysts who are aware of the fact, but the FDA *Pesticide Analytical Manual* recommends the preparation of columns to give only a single peak with endrin. DDT may be partially decomposed or partially retained in the inlet ports or on GC columns to the extent that coulometric detectors yield lower measured values for chlorine than theoretically expected. However, if the response is reproducible, quantitation with known standards is acceptable.

Under some conditions, *p,p'*-DDT may be partially converted to *p,p'*-DDE. Most unsatisfactory columns or conditions of operation are avoidable with current technology by following directions in the published methods or analytical manuals.

Detectors

Electron-capture (EC) detectors with either a tritium or nickel-63 source and with d.c. or pulsed applied voltages are commonly used, especially for halogen-containing pesticides and certain organophosphorus pesticides such as parathion and methyl parathion. They are considered the most sensitive of GC detectors for organohalogen and certain other pesticides. The tritium detectors are less expensive and the foils may be easily changed or removed for cleaning when they become contaminated. Contamination can sometimes be removed or decreased by operating the detector at its maximum permissible temperature with a higher than usual gas flow rate for periods from a few hours to a day. When there is an appreciable, permanent, irreversible decrease in standing current accompanied by a sizable decrease in response to known pesticides, the foil should be changed.

Nickel-63 detectors may be operated at high temperatures (over 300° C), thus reducing problems caused by potential condensation of contaminants in the detector. Devices are now commercially available that are capable of linearizing the response of EC detectors over several decades of concentration.

The flame-photometric detector, selective for phosphorus or sulfur, has good linearity of response for P compounds but not for S compounds. It is quite sensitive and stable in operation and is relatively free from annoying interferences.

A variety of other detectors is available for GC including: the thermionic (phosphorus, nitrogen), flame-ionization, microwave-emission, conductometric (halogen, nitrogen) and coulometric (halogen) detectors. The relative merits of such detectors for specific uses are described in various published analytical methods and manuals.

Derivatization

Derivatization is an especially useful technique for confirming the identity of pesticides; for handling relatively nonvolatile, thermally labile or highly polar compounds; or for analyzing pesticides which are relatively insensitive to other methods. Thus, although N-methylcarbamates have been difficult to analyze with high sensitivity, one derivative technique which has shown some promise involves hydrolytic cleavage and subsequent reaction of the liberated phenol with 2,4-dinitrofluorobenzene (Holden, 1973). Determination is by EC-GC.

[More direct procedures for the N-methylcarbamates using nitrogen-specific detectors for either GC or HPLC are still under investigation and evaluation (Moye, 1971).]

Quantitation

Quantitation is usually done by comparative measurement of height or area of peaks from sample and reference standards treated in the same manner. Actual measurements are made by triangulation, planimetry, disk integration, or electronic integration. In general, quantitation using peak area gives more exact results than measurements of peak heights. Disk or electronic integration is less reliable when sloping base lines or peaks with shoulders occur and must be used with extreme care. In such cases, the experienced analyst must exercise judgment to quantitate the peaks to the best of his ability. Other cleanup procedures, GC columns, or changes in the operating conditions sometimes provide improved resolution of the peaks and flatter base lines.

Peak height measurements are simple and rapid and can be used when integrators are not available and when the gas chromatograph is operating in a stable manner with high reproducibility in the retention times of the desired peaks. Peak height measurement is probably the best procedure for very narrow peaks.

OTHER DETECTION METHODS

Some other methods for detecting and measuring pesticide residues are based on spectrophotometry, fluorometry, atomic absorption, spectrometry, activation analysis, etc. Such methods, designed for individual pesticides, are often used when GC methods are not available or applicable. Many of these methods are less sensitive than GC methods and thus can be used only when relatively high pesticide residue levels occur, or in some cases, for confirmation purposes. Directions for these methods are usually well described in the literature or in manuals.

Equipment and techniques for quantitative TLC of pesticide residues have improved rapidly (Beroza, *et al.*, 1968; Hill, 1968; Getz, 1971a, b). These referenced techniques are now comparable to gas chromatography in precision, accuracy and selectivity, though generally not in sensitivity. However, in those situations where fluorescent spots are obtained or where cholinesterase inhibition can be used for visualization, they are also comparable in sensitivity. Good quantitation requires an automated plate spotter and a skilled analyst. Prepared hard-surface TLC plates which permit spot visualization by dipping instead of spraying permit better quantitation through more complete reactions. Quantitative TLC is especially useful for determining pesticides such as the carbamate insecticides, that cannot be readily analyzed by GC methods because of thermal instability.

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CHAPTER IX - Confirmation of Residue Identity

It is the responsibility of analytical laboratory personnel to support residue analyses with appropriate confirmatory work. The identity of chemical residues detected and tentatively identified by GC methods must be confirmed by tests in addition to the initial GC. It is not possible to prescribe in advance what test or combination of tests is appropriate for all sample residue situations, since a number of considerations dictate the course of action to be taken in confirming the identity of a residue. Essentially all methods and tests regularly employed in residue analysis provide qualitative information which is presumptive in nature, i.e., the behavior of an unknown is compared to that of a known standard material. It is possible in any single test to find one material that masquerades as another. When the behavior of an unknown and standard is the same in a number of tests that measure different chemical or physical properties, the certainty of identification is increased.

The importance of good qualitative interpretation in the initial GC analyses cannot be overemphasized. Two factors are necessary for obtaining reliable initial analyses: a method that has been proved to recover and detect the pesticide residues of interest, and a chemist capable of utilizing and interpreting all available information.

The analytical method is most useful when backed by an information bank of data on the behavior of many pesticides and their metabolites and degradation products in the various operations that make up the method. The chemist should be familiar with pesticide usage and the chemistry and metabolism of residues. The analyst should also be aware of the available information on the behavior of pesticides and other potential residues in the methodology used, taking into consideration the possibility of chemicals that may interfere (e.g., PCB's, phthalate esters, etc.). Most important, the analyst must be able to use common sense and make reasonable judgments in drawing analytical conclusions.

Once the interpretation of the GC records has produced a tentative residue identity, appropriate confirmatory steps must be chosen. The extent of confirmatory effort will be influenced by the significance of the sample; nature and level of the residue; and practical considerations such as time, cost, number of other samples, etc. The choice of confirmatory procedures depends on the tentative identity of the pesticide, amount of residue available for testing, sample type, and availability of instrumentation required for confirmatory tests. Some procedures often used for confirmation of residue identity, such as TLC, paper chromatography, or p-value determinations (Beroza and

Bowman, 1965; Bowman and Beroza, 1965), share the same physical property of partition in achieving separations of mixtures and therefore do not independently confirm identity (Elgar, 1971). For example, p-value determinations and TLC do not provide independent measures of a chemical's characteristics. Thus the combination of these two procedures does not add much certainty of identity. Analyses using two or more GC columns do not always assure identity except with certain pesticides on certain combinations of columns.

Some laboratories may not have time to confirm every residue tentatively identified by GC. Such laboratories must devote additional effort to samples of special importance and to residues of an unusual or significant chemical or amount. A possible alternative to confirmation of every residue is confirmation of residues in samples at intervals: one of every five samples, for example, when the same residues are apparently present throughout the group. Amounts of residue available from such samples as water or human tissues and fluids, may be inherently low, prohibiting the use of the most desirable confirmatory tests. In such instances, it may be possible to confirm residue identity on a pool of several cleaned-up sample extracts.

The choice of confirmatory tests may be limited when very low amounts of residue are available. For example, TLC may require 25 or more nanograms of an organochlorine pesticide to give a positive test. However, p-value determinations and some derivatization reactions may be employed with amounts of pesticide only slightly greater than those required for determination by EC-GC (Beroza and Bowman, 1965; Bowman and Beroza, 1965).

In view of the need for confirmation of residue identity (Elgar, 1971; Schechter, 1968; 1969) and practical limitations on confirmatory testing, the following specific guidelines on confirmation of residue identity are presented:

> Residues reported should be confirmed by tests such as TLC, element-specific GC, p-value determinations, derivatization, ultraviolet photolysis (Glotfelty, 1972; Banks and Bills, 1968), etc., in addition to EC-GC.
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> #
>
> PCB's or other suspected complex interfering substances must be separated and confirmed.
>
> #

Combinations of confirmatory tests that measure the same physical property parameters or tests that measure the same parameters as the initial analysis, although they may help to support the identification, are not the best means of doing so.

#

If sufficient material is available, excellent identifications can be made by infrared spectrometry, particularly with the use of microcell techniques or Attentuated Total Reflectance.

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Mass spectrometry (MS) or combined GC-MS is recommended for identifying or confirming identity of important residues not adequately identified by other techniques (Biros, 1971a; Safe and Hutzinger, 1973).

#

The methods used for confirming residue identity should always be described in the report of residue results.

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CHAPTER X - Sensitivity and Limit of Detection

Ambiguity exists in use of the terms *sensitivity* and *limit of detection*, especially in the analysis of pesticide residues by GC. It is important to recognize the differences in meaning (Skogerboe and Grant, 1970; Sutherland, 1965).

Instrument sensitivity denotes the response of an instrument, such as a gas chromatograph, to a given compound under a given set of instrument parameters. As the instrument sensitivity is increased, the base line noise level generally also increases. At maximum usable sensitivity, the peak height of a response to a given pesticide should be at least twice the peak-to-peak height of the noise on the chart in order to be significant.

Sensitivity may be stated in relative terms such as the recorder deflection for the peak height (in millivolts) of a given pesticide at its stated retention time, or the area of the peak (in millivolt-seconds) for a given weight of injected pesticide. If the electrometer is calibrated, these responses may be expressed in absolute units of amperes per gram injected for a peak height of a given pesticide at its stated retention time, or ampere-seconds per gram injected for a peak area.

It is sometimes desirable to operate an instrument at less than maximum sensitivity. When analyzing pesticides in food and feed, for example, FDA analysts set EC-GC instruments to give 50 percent of full-scale recorder deflection on injection of one nanogram of heptachlor epoxide or two nanograms of parathion. This means of expressing the magnitude of response provides a basis for communicating the sensitivity to personnel at other laboratories who may wish to duplicate that degree of sensitivity. In other instances, the analyst will operate an instrument at the maximum sensitivity, with due regard to noise level, in order to detect the minimum or trace amounts of residues.

In multiresidue analysis, the instrument sensitivity varies among compounds and should be determined for each compound. For optimum performance in monitoring studies, instrument sensitivity should be maintained at a fairly constant level throughout the study. Operating conditions should be checked from day to day by the use of standards and should be altered if necessary to maintain the desired sensitivity. Instrument sensitivity depends on such factors as the type, diameter, and length of column; the substrate; gas flow rates; temperatures of inlet, column, and detector; type of detector; polarizing voltage; electrometer setting, etc. These factors should be optimized to achieve the desired sensitivity.

The *limit of detection* of an analytical method, which is often confused with the sensitivity of the method, may be defined as the concentration of pesticide above which a given sample of material can be said, with a high degree of confidence, to contain the chemical analyzed (Sutherland, 1965). Because it depends upon the substrate as well as the pesticide, the limit of detection is usually given in relative units, such as parts per million (micrograms per gram of sample).

The limit of detection for an analytical method depends upon a number of factors in addition to the instrument sensitivity: sample size, adequacy of extraction and cleanup, size of aliquot injected, and knowledge and experience of the analyst. Peak or base line deviations caused by coextractives that are not removed to a sufficient extent in the cleanup procedure, are most likely to become more evident when the instrument is operated at maximum sensitivity. These deviations may cast doubt on the validity of the peaks of interest. This is especially true in monitoring programs because control samples reliably free from pesticides are usually not available to establish a good, zero-level base line. In such cases, the limit of detection should be based on the analytical variability, which should be measured at least once for each pesticide on each substrate and after any change in the method. Analytical variability can be measured by carrying through the entire method at least six replicate samples containing levels near the estimated limit of detection of the pesticide. The limit of detection should be considered equal to two standard deviations (approximately 95% confidence level) calculated from the replicated results. The identity of any peak larger than this limit should be subject to confirmation as previously discussed.

In reporting results of monitoring programs, residues below the limit of detection may be reported as *trace* (T), provided that the identity of the peak can be confirmed by pooling samples. Results below the limit of detection that cannot be confirmed, including zero, should be reported as *not detected* (ND). Ordinarily, GC peaks should be at least twice the height above the base line as the mean of the neighboring peak-to-peak base-line noise in order to be considered reportable. The limit of detection should be clearly stated for each pesticide reported. In addition to reporting the limit in relative units (ppm, ppb, etc.), the amount of sample represented in the injection and the instrument sensitivity should be given.

Practical limitations exist in the number of samples that can be analyzed and the costs involved at any given limit of detection. Generally speaking, the lower the limit of detection required for a given pesticide, the more care, time and cost will be involved in the analyses.

Unfortunately, toxicologists and biologists are often reluctant or unable to set the lower limit of biological significance for each compound so that analytical chemists can use these limits as guides in their methods.

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CHAPTER XI - Losses and Degradation of Pesticides

Losses or degradation of pesticides may occur in any step of the analyses: during partitioning of pesticides from one solvent into another; during elution of pesticides, especially the more polar ones, from a cleanup column; by volatilization of pesticides during evaporation or distillation of solutions; by overheating; by oxidation; or by photo-degradation. Some pesticides may react with solvents. A typical example is the transesterification of esters in ethyl acetate. Solutions to be analyzed as well as standards should be kept in the dark, preferably under refrigeration.

The analyst should periodically perform recovery studies as a means of demonstrating that the residues of interest are not being lost during the various steps in the analysis. Recovery studies involve the addition of a known quantity of a pesticide to a measured portion of the sample prior to the extraction step. This so-called spiked sample is analyzed in the same manner as the unspiked portion of the sample. The difference between total amount of pesticide found in the spiked sample and the amount found in the unspiked sample is the recovery.

The amount recovered should be comparable (i.e., 80-110%) to the known amount of the pesticide added. Recovery studies should be performed at different fortification levels. The results, usually expressed as percent recovery, should be included in the analyst's pesticide residue data reports.

Recovery studies demonstrate only the capability of the method for procedural steps following extraction. Such studies do not provide information on the efficiency of residue extraction from environmental samples.

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Losses and Degradation of Pesticides

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CHAPTER XII - Statistical Evaluation and Reporting of Results

ACCURACY

Accuracy involves statistical measurements that relate to the differences between the test results and the true result when the latter is known or assumed, and may be expressed as the mean error or relative error.

PRECISION

Precision involves statistical measurements that relate to the variation among test results themselves - i.e., the scatter or dispersion of a series of test results without assumption of any prior information as to the true result. Precision may be expressed in terms of the variance, standard deviation, relative standard deviation, range, etc.

MULTIPLE INJECTIONS

Although most analysts use a single injection per sample, it would be preferable to use duplicate injections per sample, or even better, single or duplicate injections from duplicate samples. The latter alternatives are not often feasible because of the heavier workloads and increased costs entailed.

COLLABORATIVE STUDIES

It is highly advisable to subject widely-used methods to collaborative study to prove their reliability, especially since inter-laboratory variations are generally greater than intra-laboratory variations. The AOAC collaborative studies are an excellent means for such collaborative work and the results usually indicate the magnitude of intra- and inter-laboratory variations. The AOAC book of methods and the *Journal* of the AOAC publish many such collaboratively tested analytical methods. *The Statistical Manual of the AOAC* (Youden and Steiner, 1975) presents excellent information on formulating and statistically analyzing such tests.

REPORTING RESULTS

When reporting results, the analyst should state:

The compounds or appropriately defined classes of compounds being sought

Other related or important compounds of interest that could have been or were detected

The limit of detection for each pesticide, as well as its degradation products and metabolites

Recovery values and whether the results have been corrected for recovery

The basis for selection of the procedure, or modifications, if different from a usual or accepted method

Confirmatory procedures.

Results should be reported in appropriate units of ppm (µg per gram), ppb, ppt, or µg per liter for water, etc. The basis for reporting results should be clearly stated: wet or dry weight; whole milk or butterfat, etc. If applicable, the method of drying samples should be described. In the case of tissue analyses, it is best to report results on both a wet-weight and fat- or extractable-lipid basis. If replicated results are reported, the individual results, the mean plus the range, or a more thorough statistical treatment should be presented as needed.

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CHAPTER XIII - Metabolites and Degradation Products

Degradation products and biologically active metabolites considered to be toxic or hazardous by toxicologists should be determined along with the pesticide, and the same analytical considerations should apply to these substances. Unless the residue method determines the major metabolites or degradation products, especially those that are toxic or biologically active, the method can be considered inadequate. To illustrate, one need only point out that metabolically derived heptachlor epoxide and dieldrin are more toxic and more frequently encountered as residues than are heptachlor and aldrin, respectively, or that the oxygen analog metabolites of many of the sulfur-containing organophosphorus insecticides are frequently much more toxic than are the original insecticides. Extraction and cleanup procedures may have to be modified to accommodate the increase in polarity of such metabolites over that of the original pesticide, with a resulting change in their solubility in solvents and elution from chromatographic columns.

When the chemistry has been elaborated and methodology is available, the analyst should attempt to detect photoalteration products, such as photodieldrin produced from dieldrin.

In the case of mercury in environmental samples, it should be kept in mind that virtually all mercury-containing pesticides and even elemental mercury itself can be converted to the highly toxic methyl mercury form by anaerobic degradation.

CHAPTER XIV - Multicomponent Pesticides and PCB's

DDT

The analytical method should be capable of determining the major isomers and metabolites of DDT, such as *p,p'*-DDT; *o,p'*-DDT; *p,p'*-DDE; and *p,p'*-TDE (*p,p'*-DDD). Even though the total residue may be reported as total DDT, DDT-complex, DDTR, etc., it is highly advisable to report individual values for the isomers and metabolites since they have different biological and toxicological properties.

CHLORDANE

The analytical method should be capable of determining the important components and metabolites of chlordane including *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, heptachlor epoxide, oxychlordane and hydroxychlordene. Here took it is advisable to report individual values if possible.

TOXAPHENE AND STROBANE

Because relative heights of the peaks may change with weathering or metabolism, toxaphene and strobane residues are difficult to quantitate by GC. The simplest procedure is to integrate gas chromatograms over the area in which the compounds emerge for both the samples and reference standards. Spectrophotometric methods are available, although only for residue levels higher than those detectable by GC.

POLYCHLORINATED BIPHENYLS (PCB's)

The polychlorinated biphenyls (or polychlorobiphenyls) are a group of industrial chemicals manufactured in this country by Monsanto Chemical Company under the trade name Aroclor^{R}. They are complex mixtures of chlorinated biphenyls which have been used as transformer oils, condenser dielectrics, plasticizers, printing ink components, and for many other industrial purposes. They are very stable and persistent and, like DDT, are widely distributed in the environment, particularly in marine life. In GC, the PCB's exhibit multiple peaks, some of which coincide with or overlap the peaks of some organochlorine pesticides, hence they can cause confusion in identification and quantitation of such pesticides. Because of the analytical complications caused by PCB's in environmental samples, the analyst should be able to select appropriate procedures for separating PCB's from organochlorine pesticides, for correctly identifying peaks attributable to each group

of compounds, and for the most desirable method of quantifying the PCB's (ACS, 1972; Berg *et al.*, 1972; Armour, 1973). Perchlorination with antimony pentachloride has proved useful in confirming PCB residues (Berg, *et al.* 1972; Huckins *et al.*, 1974).

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CHAPTER XV - Reference Standards

There are several sources of pesticide standards. Most manufacturing companies are willing to supply a sample of the technical grade of pesticide, and in some instances, a purified grade sufficiently good for most analytical purposes. The company's analytical department may be able to supply a highly purified analytical sample, but usually only in very small amounts.

The analyst may sometimes prepare his own standard by repeatedly recrystallizing the technical material until high purity is achieved, as determined by measurements of two or more criteria such as elemental composition, infrared spectrum, melting point, GC trace, etc.

The EPA Quality Assurance Program maintains a pesticide calibration and reference materials repository at its Pesticide and Toxic Substances Laboratory at Research Triangle Park, N. C. This laboratory supplies materials to other nonprofit, government, and university laboratories on a discretionary basis as time and resources permit. Because of great demand from many sources and limited supplies, the amount of standard sent out is limited to no more than 100 mg and the number of standards to only those necessary for limited immediate needs.

The Pesticide Reference Standards Section, Chemistry Branch, Registration Division, EPA, Washington, D. C., has a program of preparation of pure primary standards of pesticides and metabolites and other standards of high purity as well as important derivatives for pesticide residue analysis. These standards, accompanied by data sheets, are available on a limited and restricted basis, in amounts of 100 mg or less, to major central laboratories.

The National Physical Laboratory, Ministry of Technology, Chemical Standards, Teddington, Middlesex, England, sells highly purified pesticide reference standards to any interested party. The laboratory welcomes orders for these materials, using the income to support their program.

Pesticide standards and kits are also available from a number of companies handling gas chromatographic equipment and supplies.

CHAPTER XVI - Manuals and General References

MANUALS

Several federal, state, and foreign government agencies have compiled or published manuals of pesticide analytical methods applicable to their specific areas of interest. There are also a number of professional-nongovernmental and commercial organizations which produce such manuals. The following paragraphs identify the more widely referenced manuals with appropriate notes on their applicability, availability and contents.

Pesticide Analytical Manual, (2 Vols.) 1968, Food and Drug Administration, U. S. Dept. of Health, Education and Welfare, Washington, D. C. (looseleaf). Volume I (2nd Edition, 1968 and subsequent revisions) contains directions and associated background information for multiresidue methods used by FDA to analyze food and feed samples collected under its surveillance programs. Volume II (1967 and subsequent revisions) contains methods which can be used to analyze for single compounds. Each volume is revised continuously to reflect appropriate changes and additions to the methodology and information.

The Identification and Measurement of Chlorinated Hydrocarbon Pesticides in Surface Waters. 1968, A. W. Breidenbach, J. J. Lichtenberg, C. F. Henke, D. J. Smith, J. W. Eichelberger, Jr., and H. Stirell. Federal Water Pollution Control Administration, USDI, Washington, D. C., 70 pp.

Methods for Organic Pesticides in Water and Wastewater. 1971, J. J. Lichtenberg, compiler. EPA, National Environmental Research Center, Cincinnati, Ohio, 64 pp. This manual revised the older 31-page publication of the *FWPCA Method for Chlorinated Hydrocarbon Pesticides in Water and Watewater* published in 1969 by the USDI, Federal Water Pollution Control Administration, Analytical Control Laboratory, Cincinnati, Ohio.

Manual of Chemical Methods for Pesticides and Devices. 1975, Chemical and Biological Investigations Branch, OPP/TSD. Environmental Protection Agency, Washington, D. C. (looseleaf). This two volume manual contains: a cross-reference index of pesticide names, methods of analysis, techniques of analysis (good practices), references, infrared spectra of pesticides, and tables of accepted names, structures and solubilities.

Manual of Analytical Methods. Analysis of Pesticide Residues in Human and Environmental Samples. 1972, J. F. Thompson, Editor. EPA, National Environmental Research Center, Research Triangle Park, N. C. (looseleaf).

Laboratory Manual. Pesticides Monitoring Laboratory. 1975, Report PML-2, Pesticides Monitoring Laboratory. EPA, Bay St. Louis, Miss., (draft, Feb. 5, 1975) 122 pp. This draft report is marked for internal use only.

Handbook of Procedures for Pesticide Residue Analysis. 1972, R. C. Tindle, Compiler. Technical Papers of the Bureau of Sport Fisheries and Wildlife No. 65, U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, D. C., 88 pp.

Patuxent Analytical Manual for Organochlorine Compounds. W. L. Reichel, compiler. U. S. Department of the Interior, Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. (mimeo). This manual was developed for internal use at the Patuxent Wildlife Research Center.

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Analytical Methods for Pesticide Residues in Foods. 1969, compiled by H. A. McLeod, P. J. Wales, R. A. Graham, M. Osadchuk and N. Bluman. Department of National Health and Welfare, Food and Drug Directorate, Ottowa, Canada (looseleaf).

Pesticide Manual. 1972, 3rd Edition, H. Martin, Editor. British Crop Protection Council, Worcester, England, 495 pp. This manual lists pesticides by physical-chemical descriptions and provides brief toxicological information and references to analytical methods.

Pesticide Residue Analytical Manual. 1965, B. Munks. Florida Department of Agriculture, Tallahassee, Fla., 1211 pp. This is an example of manuals developed and used by some state agencies for official analyses in the individual states.

Official Methods of Analysis of the Association of Official Analytical Chemists, 12th Ed. 1975, W. Horwitz, Editor. Association of Official Analytical Chemists, Washington, D. C., 1015 pp. This manual is revised every four years and annual supplements of changes in the official methods are issued in March of the intervening years.

Herbicide Handbook of the Week Science Society of America, 3rd Edition. 1974. Weed Science Society of America, Champaign, Ill., 430 pp. This manual lists approximately 170 herbicides, desicants, plant growth regulators, etc., by the common, chemical, and trade names and provides brief statements of general synthesis and analytical methods.

A Guide to the Analysis of Pesticides by Gas Chromatography, 2nd Edition. 1968, A. T. Preston. Polyscience Corp., Evanston, Ill.

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